In accordance with 37 C.F.R. § 1.821(f) and (g), attached hereto is a signed statement verifying that the content of the paper and CRF copies of the Sequence Listing form submitted herewith are the same, and that the submitted Sequence Listing contains no new matter.

IN THE SPECIFICATION:

Please substitute the paragraph beginning at line 14 of page 5 with the following amended paragraph:

- - Wolfe et al. (*Theriogenology*, 33:350 (1990)) describe fusing bovine embryonic cells with oocytes of other mammalian species to produce cross species nuclear transfer (NT) units, some of which divided to produce embryonic structures possibly having an inner cell mass. No inner cell mass structures were obtained when cattle nuclei were fused into hamster oocytes during NT. Wolfe et al. did not describe attempts to use non-embryonic cells, e.g., adult cells, as donor nuclei in the nuclear transfer procedure. The dogma has been that embryonic cells are more easily reprogrammed than adult cells. This dates back to earlier NT studies in the frog (review by DiBerardino, *Differentiation*, 17:17-30 (1980)). Wolfe et al. also did not report that the inner cell mass cells from NT units could be used to form an ES cell-like colony that could be propagated. - -

Please substitute the paragraph beginning at line 17 of page 14 with the following amended paragraph:

- - The present discovery was made based on the observation that nuclear transplantation of the nucleus of an adult human cell, specifically a human epithelial cell obtained from the oral cavity of a human donor, when transferred into an enucleated bovine oocyte, resulted in the formation of nuclear transfer units, the cells of which upon culturing gave rise to human stem-like or embryonic cells and human embryonic or stem-like cell colonies. This result has

recently been reproduced by transplantation of keratinocytes from an adult human into an enucleated bovine oocyte with the successful production of a blastocyst and ES cell line. Based thereon, it is hypothesized by the present inventors that bovine oocytes and human oocytes, and likely mammals in general must undergo maturation processes during embryonic development which are sufficiently similar or conserved so as to permit the bovine oocyte to function as an effective substitute or surrogate for a human oocyte. Apparently, oocytes in general comprise factors, likely proteinaceous or nucleic acid in nature, that induce embryonic development under appropriate conditions, and these functions are the same or very similar in different species. These factors may comprise material RNAs and/or telomerase. - -

Please substitute the paragraph beginning at line 7 of page 23 with the following amended paragraph:

- - Activated NT units may be cultured in a suitable *in vitro* culture medium until the generation of embryonic or stem-like cells and cell colonies. Culture media suitable for culturing and maturation of embryos are well known in the art. Examples of known media, which may be used for bovine embryo culture and maintenance, include Ham's F-10 + 10% fetal calf serum (FCS), Tissue Culture Medium-199 (TCM-199) + 10% fetal calf serum, Tyrodes-Albumin-Lactate-Pyruvate (TALP), Dulbecco's Phosphate Buffered Saline (PBS), Eagle's and Whitten's media. One of the most common media used for the collection and maturation of oocytes is TCM-199, and 1 to 20% serum supplement including fetal calf serum, newborn serum, estrual cow serum, lamb serum or steer serum. A preferred maintenance medium includes TCM-199 with Earl salts, 10% fetal calf serum, 0.2 mM Na pyruvate and 50 μg/ml gentamicin sulphate. Any of the above may also involve co-culture

Q3 with a variety of cell types such as granulosa cells, oviduct cells, BRL cells, uterine cells and STO cells. - -

Please substitute the paragraph beginning at line 3 of page 28 with the following amended paragraph:

- - Another preferred embodiment of the present invention is the production of nuclear transfer embryos that grow more efficiently in tissue culture. This is advantageous in that it should reduce the requisite time and necessary fusions to produce ES cells and/or offspring (if the blastocysts are to be implanted into a female surrogate). This is desirable also because it has been observed that blastocysts and ES cells resulting from nuclear transfer may have impaired development potential. While these problems may often be alleviated by alteration of tissue culture conditions, an alternative solution is to enhance embryonic development by enhancing expression of genes involved in embryonic development. - -

Q4

Please substitute the paragraph beginning at line 1 of page 31 with the following amended paragraph:

- - Cyclins are proteins that are expressed only during specific stages of the cell cycle. They include cyclin D1, D2 and D3 in GI phase, cyclin B1 and B2 in G2/M phase and cyclin E, A and H in S phase. These proteins are easily translated and destroyed in the cytosol. This "transient" expression of such proteins is attributable in part to the presence of a "destruction box", which is a short amino acid sequence that is part of the protein that functions as a tag to direct the prompt destruction of these proteins via the ubiquitin pathway. (Adams et al, *Science,* 281 (5321):1322-1326 (1998)). - -

Q5

Please substitute the paragraph beginning at line 28 of page 31 with the following amended paragraph:

-- Yet another means for enhancing the development of nuclear transfer embryos *in vitro* is by optimizing culture conditions. One means of achieving this result will be to culture NT embryos under conditions impede apoptosis. With respect to this embodiment of the invention, it has been found that proteases such as caspases can cause oocyte death by apoptosis similar to other cell types. *(See,* Jurisicosva et al, *Mol. Reprod. Devel., 51(3):243-253* (1998).) --

Please substitute the paragraph beginning at line 5 of page 32 with the following amended paragraph:

-- It is anticipated that blastocyst development will be enhanced by including in culture media used for nuclear transfer and to maintain blastocysts or culture preimplantation stage embryos one or more caspase inhibitors. Such inhibitors include by way of example caspase-4 inhibitor I, caspase-3 inhibitor I, caspase-6 inhibitor II, caspase-9 inhibitor II, and caspase-1 inhibitor I. The amount thereof will be an amount effective to inhibit apoptosis, e.g., 0.00001 to 5.0% by weight of medium; more preferably 0.01 % to 1.0% by weight of medium. Thus, the foregoing methods may be used to increase the efficiency of nuclear transfer by enhancing subsequent blastocyst and embryo development in tissue culture. --

Please substitute the paragraph beginning at line 14 of page 32 with the following amended paragraph:

-- After NT units of the desired size are obtained, the cells are mechanically removed from the zona and are then used to produce embryonic or stem-like cells and cell lines. This is preferably effected by taking the clump of cells which comprise the NT unit, which typi-

cally will contain at least about 50 cells, washing such cells, and plating the cells onto a feeder layer, e.g., irradiated fibroblast cells. Typically, the cells used to obtain the stem-like cells or cell colonies will be obtained from the inner most portion of the cultured NT unit which is preferably at least 50 cells in size. However, NT units of smaller or greater cell numbers as well as cells from other portions of the NT unit may also be used to obtain ES-like cells and cell colonies. - -

Q 8

Please substitute the paragraph beginning at line 1 of page 35 with the following amended paragraph:

- - Moreover, Pedersen, *J. Reprod. Fertil. Dev.*, 6:543-552 (1994) is a review article which references numerous articles disclosing methods for *in vitro* differentiation of embryonic stem cells to produce various differentiated cell types including hematopoietic cells, muscle, cardiac muscle, nerve cells, among others. - -

Q 9

Please substitute the paragraph beginning at line 6 of page 37 with the following amended paragraph:

- - Also, human embryonic or stem-like cells produced according to the invention may be used to produce genetically engineered or transgenic human differentiated cells. Essentially, this will be effected by introducing a desired gene or genes, which may be heterologous, or removing all or part of an endogenous gene or genes of human embryonic or stem-like cells produced according to the invention, and allowing such cells to differentiate into the desired cell type. A preferred method for achieving such modification is by homologous recombination because such technique can be used to insert, delete or modify a gene or genes at a specific site or sites in the stem-like cell genome. - -

Q 10

Please substitute the paragraph beginning at line 25 of page 45 with the following amended paragraph:

- - Mitochondrial DNA was analyzed using two independent methods. 1.) *Restriction Fragment Polymorphism.* Approximately 0.25 μg of total DNA extracted from different tissues by standard procedures (Moraes, 1992) were used to amplify a 483 bp fragment from the mtDNA D-loop region. Oligonucleotide sequences corresponded to positions 16021-16043 and 165-143 of the *Bos taurus* mitochondrial genome (GENBANK accession number NC_001567) (Anderson et al., 1982). Although there is variation in the nucleotide sequence between the *Bos taurus* and *Bos gaurus* (GENBANK accession number AF083371) (Ward et al, 1999) mtDNA D-loop regions, the oligonucleotide primers have 100% homology with mtDNA from both species. The amplified fragment was labeled with [^{32}P]-dCTP in the last cycle of the PCR to avoid the detection of heteroduplexes (Moraes et al, 1992). Amplicons were digested with *Sph*I or *Scr*FI and analyzed by PAGE and Phosphorimaging (Cyclone, Packard Inst.). *2) Allele Specific PCR.* Oligonucleotide primers corresponding to relatively divergent regions of the mtDNA D-loop were used to amplify a 480 bp fragment specifically from *taurus* or *gaurus*. The *gaurus* primers were:

forward CATAGTACATGAACTCATTAATCG (SEQ ID NO: 1) and

reverse TTGACTGTAATGCCCATGCC (SEQ ID NO: 2). The *taurus* primers were:

forwardCATAATACATATAATTATTGACTG (SEQ ID NO: 3) and

reverse TTGACTGTAATGTCCATGCT (SEQ ID NO: 4). Amplifications were performed with the following cycling program: 94°C 1' : 65°C 1' : 72°C 1' for 30 cycles. Microsatellite analysis of the bovine chromosome 21 (D21 S 18, Research Genetics) was performed by PCR amplification of the marker after end-labeling one of the oligonucleotide